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ABSTRACT

This pamphlet is the seventh in a series of nine discussing the Apollo-Soyuz mission and experiments. This set is designed as a curriculum supplement for secondary and college teachers, supervisors, curriculum specialists, textbook writers, and the general public. These booklets provide sources of ideas, examples of the scientific method, references to standard textbooks, and descriptions of space experiments. There are numerous diagrams, as well as questions for discussion (with answers) and a glossary of terms. This booklet describes fish in zero-g, microbial growth in zero-g, separation of cells by electrophoresis, microbes at large in the spacecraft, and changes in astronaut immunity during spaceflight. (MA)

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Apollo-Soyuz Pamphlet No. 7:

Biology in Zero-G

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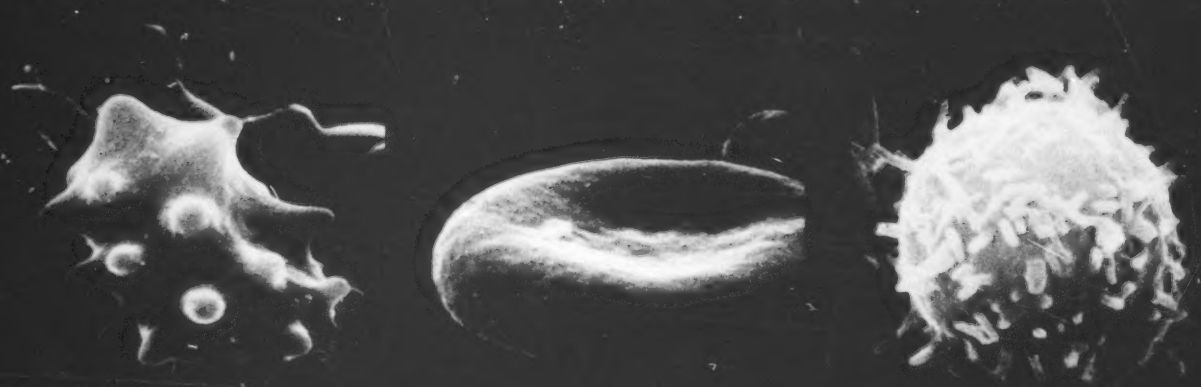
Polymorphonuclear leukocyte
· 2500

Polymorphonuclear leukocyte
and [illegible] sp. (yeast cells)
· 3600

[illegible] cells
· 6000



Crenated red blood cell
· 13 000

Red blood cell
· 13 000

Lymphocyte
· 15 000

SE 025 187

Apollo-Soyuz Experiments In Space

This is one of a series of nine curriculum-related pamphlets for Teachers and Students of Space Science

Titles in this series of pamphlets include:

EP-133 Apollo-Soyuz Pamphlet No. 1 The Flight

EP-134 Apollo-Soyuz Pamphlet No. 2 X-Ray Gamma Ray

EP-135 Apollo-Soyuz Pamphlet No. 3 Sun Stars and Zero-G

EP-136 Apollo-Soyuz Pamphlet No. 4 Ground and Flight

EP-137 Apollo-Soyuz Pamphlet No. 5 Experiments Other

EP-138 Apollo-Soyuz Pamphlet No. 6 Cosmic Ray Detector

EP-139 Apollo-Soyuz Pamphlet No. 7 Biology and Zero-G

EP-140 Apollo-Soyuz Pamphlet No. 8 Zero-G Technology

EP-141 Apollo-Soyuz Pamphlet No. 9 Ground Support

On The Cover

Scanning Electron Micrographs of Cells
Cellular Analysis Laboratory
NASA Lyndon B. Johnson Space Center

Apollo-Soyuz Pamphlet No. 7:

Biology in Zero-G

Prepared by Lou Williams Page and Thornton Page From Investigators' Reports of Experimental Results and With the Help of Advising Teachers

NASA

National Aeronautics and Space Administration

Washington, D.C. 20546
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Preface

The Apollo-Soyuz Test Project (ASTP), which flew in July 1975, aroused considerable public interest; first, because the space rivals of the late 1950's and 1960's were working together in a joint endeavor, and second, because their mutual efforts included developing a space rescue system. The ASTP also included significant scientific experiments, the results of which can be used in teaching biology, physics, and mathematics in schools and colleges.

This series of pamphlets discussing the Apollo-Soyuz mission and experiments is a set of curriculum supplements designed for teachers, supervisors, curriculum specialists, and textbook writers as well as for the general public. Neither textbooks nor courses of study, these pamphlets are intended to provide a rich source of ideas, examples of the scientific method, pertinent references to standard textbooks, and clear descriptions of space experiments. In a sense, they may be regarded as a pioneering form of teaching aid. Seldom has there been such a forthright effort to provide, directly to teachers, curriculum-relevant reports of current scientific research. High school teachers who reviewed the texts suggested that advanced students who are interested might be assigned to study one pamphlet and report on it to the rest of the class. After class discussion, students might be assigned (without access to the pamphlet) one or more of the "Questions for Discussion" for formal or informal answers, thus stressing the application of what was previously covered in the pamphlets.

The authors of these pamphlets are Dr. Lou Williams Page, a geologist, and Dr. Thornton Page, an astronomer. Both have taught science at several universities and have published 14 books on science for schools, colleges, and the general reader, including a recent one on space science.

Technical assistance to the Pages was provided by the Apollo-Soyuz Program Scientist, Dr. R. Thomas Giuli, and by Richard R. Baldwin, W. Wilson Lauderdale, and Susan N. Montgomery, members of the group at the NASA Lyndon B. Johnson Space Center in Houston which organized the scientists' participation in the ASTP and published their reports of experimental results.

Selected teachers from high schools and universities throughout the United States reviewed the pamphlets in draft form. They suggested changes in wording, the addition of a glossary of terms unfamiliar to students, and improvements in diagrams. A list of the teachers and of the scientific investigators who reviewed the texts for accuracy follows this Preface.

This set of Apollo-Soyuz pamphlets was initiated and coordinated by Dr. Frederick B. Tuttle, Director of Educational Programs, and was supported by the NASA Apollo-Soyuz Program Office, by Leland J. Casey, Aerospace Engineer for ASTP, and by William D. Nixon, Educational Programs Officer, all of NASA Headquarters in Washington, D.C.

Appreciation is expressed to the scientific investigators and teachers who reviewed the draft copies; to the NASA specialists who provided diagrams and photographs; and to J. K. Holcomb, Headquarters Director of ASTP operations, and Chester M. Lee, ASTP Program Director at Headquarters, whose interest in this educational endeavor made this publication possible.

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1 Introduction

After 4 years of preparation by the U.S. National Aeronautics and Space Administration (NASA) and the U.S.S.R. Academy of Sciences, the Apollo and Soyuz spacecraft were launched on July 15, 1975. Two days later at 16:09 Greenwich mean time on July 17, after Apollo maneuvered into the same orbit as Soyuz, the two spacecraft were docked. The astronauts and cosmonauts then met for the first international handshake in space, and each crew entertained the other crew (one at a time) at a meal of typical American or Russian food. These activities and the physics of reaction motors, orbits around the Earth, and weightlessness (zero-g) are described more fully in Pamphlet I, "The Spacecraft, Their Orbits, and Docking" (EP-133).

Thirty-four experiments were performed while Apollo and Soyuz were in orbit: 23 by astronauts, 6 by cosmonauts, and 5 jointly. These experiments in space were selected from 161 proposals from scientists in nine different countries. They are listed by number in Pamphlet I, and groups of two or more are described in detail in Pamphlets II through IX (EP-134 through EP-141, respectively). Each experiment was directed by a Principal Investigator, assisted by several Co-Investigators, and the detailed scientific results have been published by NASA in two reports: the Apollo-Soyuz Test Project Preliminary Science Report (NASA TM X-58173) and the Apollo-Soyuz Test Project Summary Science Report (NASA SP-412). The simplified accounts given in these pamphlets have been reviewed by the Principal Investigators or one of the Co-Investigators.

For biological experiments, the major advantage in the Apollo-Soyuz spacecraft was weightlessness, or zero-g. Another feature that made the spacecraft different from ground-based laboratories was the cosmic-ray flux—a bombardment of high-energy ions, discussed in Pamphlet VI. Zero-g was known to affect humans in several ways. How would it affect smaller living organisms? How could it be used to improve biological techniques? In addition, the small, sealed spaces where the three astronauts and two cosmonauts lived for 6 to 9 days provided ideal conditions for experiments with the microbes that live in and on human beings. The seven biological experiments on Apollo-Soyuz were all concerned with a larger question: what happens to living organisms on long flights in space?

Experiment MA-161, Killifish Hatching and Orientation, observed the effects of zero-g on fish eggs and small fish. The Principal Investigator was H. W. Scheld of the Baylor College of Medicine in Houston, Texas. He was assisted by 11 Co-Investigators from five universities in the United States and one in West Germany.

Experiment MA-147, Zone-Forming Fungi, was a joint American-Soviet experiment on the growth rate of microorganisms in zero-g. The American Principal Investigators were G. R. Taylor and T. D. Rogers of the NASA Lyndon B. Johnson Space Center (JSC) in Houston, Texas. The Russian

Principal Investigator was I. G. Akoev of the Soviet Institute of Biological Physics in Pushino, near Moscow.

Experiment MA-011, Electrophoresis Technology, tested the static-column technique for separating blood cells and kidney cells in zero-g. The Principal Investigator was R. E. Allen of the NASA George C. Marshall Space Flight Center (MSFC) in Huntsville, Alabama. He was assisted by 13 Co-Investigators from several universities and commercial companies.

Experiment MA-014, Electrophoresis, tested the free-flow electrophoresis technique for separating blood cells in zero-g. The Principal Investigator, K. Hannig, and one Co-Investigator were from the Max Planck Institute of Biochemistry in Munich, Germany.

Experiment AR-002, Microbial Exchange, another joint experiment, checked the transfer and multiplication of microbes in Apollo and Soyuz. The American Principal Investigator was G. R. Taylor of JSC, who was assisted by biologists from several U.S. laboratories. The Russian Principal Investigator was S. N. Zaloguev of the Institute of Biomedical Problems, U.S.S.R. Ministry of Health, Moscow.

Experiment MA-031, Cellular Immune Response, detected changes in astronaut immunity during the Apollo-Soyuz mission. The Principal Investigator was B. Sue Criswell of the Baylor College of Medicine in Houston.

Experiment MA-032, Effects of Spaceflight on Polymorphonuclear Leukocyte Response, checked leukocytes in the astronauts' blood during and after the mission. The Principal Investigator was R. R. Martin of the Baylor College of Medicine.

The cosmonauts also performed three biological experiments in which the astronauts were not involved (see Pamphlet I)

(1) An experiment on the growth and mobility of the bacteria *Protea vulgaris* studied the results of adding various nutrients to a culture in zero-g.

(2) An experiment on the growth of *Danyo Rerio* fish in zero-g was similar to Experiment MA-161.

(3) An experiment on the genetic effect of zero-g on cell division and sprouting seeds involved observations of *Chlamydomonada* zygotes and two species of seeds.

Ground-control specimens were maintained for all the Apollo-Soyuz biological experiments either in the United States or the U.S.S.R. or in both countries.

2 "Up" and "Down" for Fish in Zero-g

There is no "up" and "down" in an orbiting spacecraft: loose objects and astronauts "float" around weightlessly, and water will not stay in an ordinary cup. During the early part of a flight, this zero-g condition is confusing to astronauts and cosmonauts, but after a day or so, they adapt to weightlessness.

Similar adaptation was observed in two small fish taken along on the Skylab 3 mission. The fish were carried in seawater in a plastic bag fastened to a locker door. During the first 2 or 3 days of weightlessness, they swam rapidly in loops and circles, obviously confused. In their normal shallow water on Earth at one-g, these minnows would remain horizontal, with their backs up and bellies down. After about 3 days in Skylab, they decided that the dark locker door was "down" and swam mostly with their bellies facing the door. The minnows required slightly more time to adapt to zero-g than the astronauts did.

The MA-161 Experiment on Apollo-Soyuz was designed to investigate this adaptation further, using the same strain of minnow ("killifish," *Fundulus heteroclitus* from Beaufort, North Carolina) and its unhatched eggs. This particular species of fish has been studied extensively by biologists, and the manner of its development from eggs into embryos[1] is well known. In particular, the biologists know at what time each part of the embryo starts to develop. One part of importance in this study was the vestibular organ in each ear, which controls an individual's balance and sense of which way is down. There are three small calcium "earstones" or "otoliths" in the vestibula on each side of the head. They start to form 66 hours after the egg is fertilized. The vestibular organ is developed by 128 hours and functioning after 216 hours. After 336 hours (14 days) the egg hatches, producing a "hatchling" about 1 centimeter long. The hatchling is mostly head; the body and tail are very small. Three or four weeks later, the new fish is 2 or 3 centimeters long and is called a "juvenile." In another few months, the adult minnow is about 10 centimeters long, fully grown.

A Plan for the MA-161 Experiment, Killifish Hatching and Orientation

The objective of Experiment MA-161 was to discover whether zero-g affects the development of an embryo in such a way that a minnow, when hatched, is

[1]BSCS-Y, Ch. 29; BSCS-B, Ch. 15. (Throughout this pamphlet, references will be given to key topics covered in these two standard textbooks: "Biological Science: An Inquiry Into Life," Biological Sciences Curriculum Study, Yellow Version (BSCS-Y), third edition, Harcourt, Brace, and Jovanovich, 1973, and "Biological Science: Molecules to Man," Biological Sciences Curriculum Study, Blue Version (BSCS-B), Houghton Mifflin Co., 1973.)

not confused because there is "no up or down." If the development of earstones were retarded or somehow changed, the hatchling would have no sense of "down" and would not be confused by zero-g. Scientists also suspected that zero-g might affect the development of the vestibula and possibly the little otoliths inside it. Formation of the otoliths involves calcium deposits in the embryo. A loss of calcium had previously been observed in astronauts after long spaceflights, and this effect on humans might be duplicated in the development of the fish embryos.

Because the Apollo-Soyuz flight was only 9 days long and *Fundulus heteroclitus* (killifish) minnow eggs take 15 days or more to hatch, it was necessary to fly five batches of eggs, each at a different stage of development. These stages are listed in Table 2.1. The eggs were carried in five separate compartments of a plastic bag filled with seawater. Each batch had been carefully prepared (fertilized at the right time, stored in seawater, and washed regularly), and there were two equal-sized control groups. One control group (C-I) was flown with the flight group to the Apollo launch site at the NASA John F. Kennedy Space Center (KSC) in Florida, where it stayed on the ground but went through the same environmental changes (with the exception of weightlessness) as occurred in the cabin atmosphere on Apollo-Soyuz. A second control group (C-II) remained in Houston at normal atmosphere and constant temperature (295 K, 22° C, or 72° F). A smaller control sample was killed and "fixed" with chemical preservative at the time of launch. This group recorded the state of development at launch for each batch of eggs. A fourth control group (C-III) contained five batches of eggs that were 48 hours younger than the five batches of flight eggs. These eggs went through the launch vibrations in a simulator at JSC 48 hours after the actual launch to test the effects of the launch "shakeup." In addition, these C-III eggs were subjected to the changes in temperature and pressure experienced by the eggs in the flight group on Apollo-Soyuz.

A second plastic bag carried six 21-day-old juvenile minnows in each of five compartments filled with seawater, as listed in Table 2.1(b). These five batches of fish had each been "preconditioned" by different visual surroundings. The purpose of this part of the MA-161 Experiment was to test the influence of *light* on the minnows' sense of up-and-down. (The Skylab minnows chose the dark cabinet door as "down.") Previous experiments in one-g on Earth showed that if a bright light were directed horizontally at a minnow in an otherwise dark pool of water, the minnow would lean about 45° toward the light. The minnow expected the light to come from the sky above the pool and used its *eyes* as well as its vestibular sense of up-and-down to keep itself upright. When zero-g eliminated the vestibular sense, the fish adapted to using its eyes only.

(a) Egg package with five compartments

Compartment	*Number of eggs*		*Age of embryos at launch, hr (a)*	*Nominal stage of embryogenesis*
	Launch	*Return*		
1	100	100	32	Midgastrulation
2	100	100	66	Statolith precursors appear
3	100	100	128	Vestibular development completed
4	100	100	216	Vestibular system functional
5	50	50	336	Full development; able to hatch

(b) Fish package with five compartments

Compartment	*Number of fish*		*Preconditioning*	*Flight background*
	Launch (b)	*Return*		
1	6	5	Neutral background	White
2	6	5	Vertical bars	Barred
3	6	5	Horizontal bars	Barred
4	6	[c]0	Black overhead	Black
5	6	6	Blinded	White

[a]Development occurred at a constant temperature of 295 K (22° C, 72° F).
[b]Age of fish at launch was approximately 21 days.
[c]All fish in this compartment were dead.

Other experiments on minnows' orientation in one-g are shown in Figure 2.1. Minnows were put in the water inside a slowly rotating drum, one at a time. The drum had black and white stripes painted on the inside. When the drum was vertical, as on the left side of Figure 2.1, the minnow inside would swim along with one of the stripes. When the drum was horizontal, as on the right side of Figure 2.1, the minnow would roll or tilt along the direction of

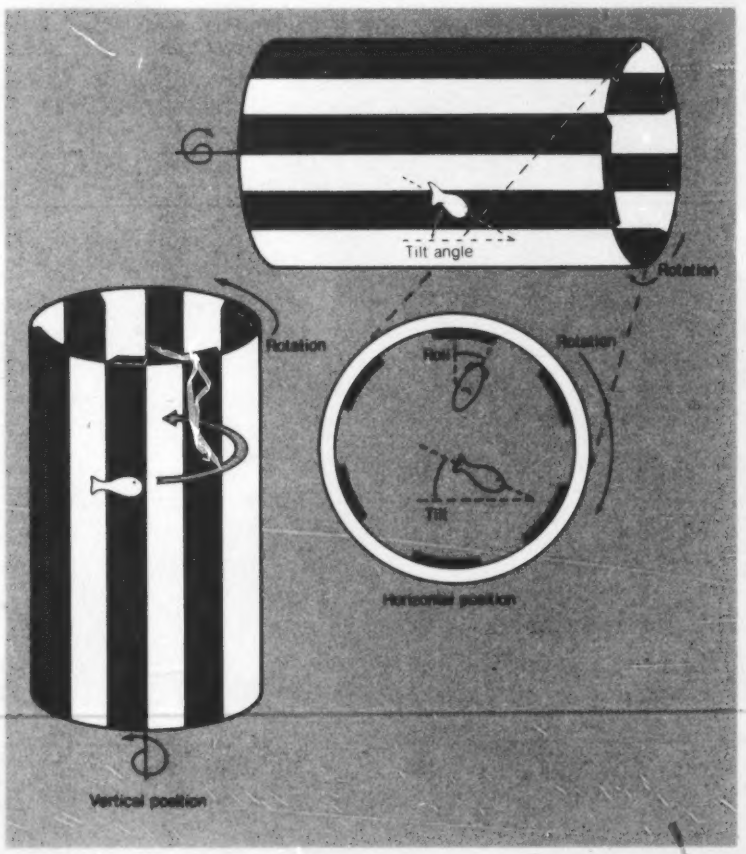


Figure 2.1 **Minnows in rotating drums. In the vertical drum, the fish would swim along beside one of the stripes. In the horizontal drum, the fish would roll or tilt in the direction of rotation.**

rotation. Two batches (nos. 2 and 3) of juvenile minnows for the MA-161 Experiment were brought up in a striped world, one batch with vertical stripes and the other with horizontal stripes. Their compartments in the plastic bag filled with seawater were fastened to a striped background in the flight package (Fig. 2.2). Another batch (no. 1) was brought up in water tanks with white walls, and another batch (no. 4) was grown in tanks with black overhead. The fifth batch of fish was blinded just after hatching so that their eyes could not be used to keep themselves upright.

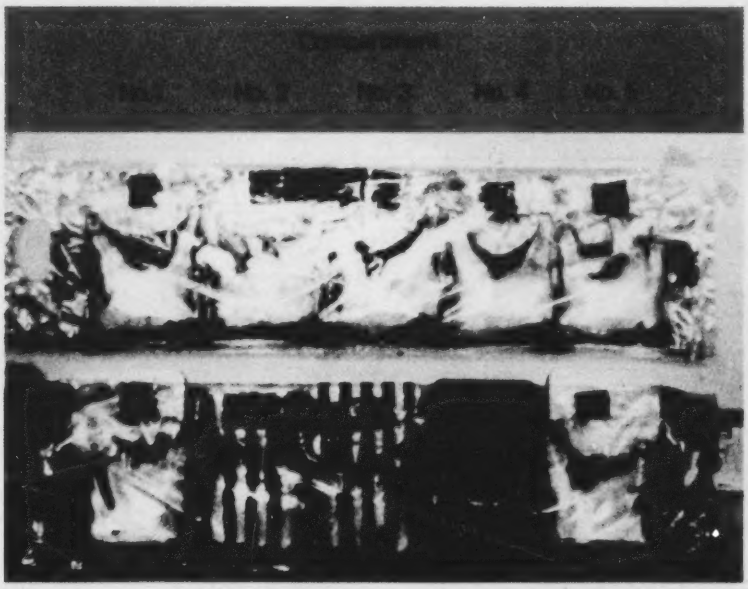


Figure 2.2 **Experiment MA-161 flight package of fish and egg packets. The five plastic compartments in the top row contained 50 or 100 eggs each in saltwater. The lower five compartments, with gray, striped, or black backgrounds, contained six minnows each.**

B Experiment MA-161 on Apollo-Soyuz

The open flight package with the five compartments of eggs at the top and the five compartments of fish at the bottom is shown in Figure 2.2. The striped background can be seen behind fish Compartments 2 and 3. This package was

fastened to a wall of the Docking Module (DM; see Pamphlet I) on the second day of the flight. Motion pictures of the fish compartments were taken on Day 2, Day 3, and Days 6 through 9. An astronaut watched the fish for several minutes each day and reported how they were swimming. Ten of the eggs in Compartment 5 hatched during flight. On Day 9, the astronauts tried to get the minnows to roll with their backs toward a light in zero-g. The lights in the DM were turned out, and a floodlight was shined on the fish compartments at a 45° angle while photographs were taken. The minnows in Compartments 1, 2, and 3, now convinced that the cabin wall was "down," refused to roll.

The entire package was returned to the Apollo Command Module (CM; see Pamphlet I) and subsequently unloaded on the recovery ship U.S.S. *New Orleans* 8 hours after splashdown. There the compartments were photographed again. Egg Compartment 5 was put in a shaker, which caused the rest of the eggs to hatch, and movies were taken of the little hatchlings swimming. Then two each of the eggs, hatchlings, and juveniles in all compartments were killed and "fixed" with chemical preservative so that they could later be dissected and examined under microscopes. These samples and the batches of live eggs and fish in new containers filled with fresh seawater were flown to JSC in Houston for further tests.

The astronauts reported that the juvenile minnows swam in loops and circles early in flight but that most of them seemed to have adapted to zero-g 5 or 6 days later. The fish in Compartments 2, 3, and 4 faced the wall unless their plastic bag was disturbed. The blinded fish in Compartment 5 and the fish in Compartment 1 against the white wall continued to loop, but more slowly. The 10 hatchlings in egg Compartment 5 sometimes swam in loops during the flight, but all the fish swam normally when first observed in one-g on the U.S.S. *New Orleans*. This shows that their vestibular organs were working well.

C Postflight MA-161 Tests and Dissections

Tests in rotating drums (Fig. 2.1) were begun on the U.S.S. *New Orleans* and continued for several days. No change from preflight behavior was observed. Two days after splashdown, tests with lights were started at JSC in Houston and continued for several weeks. All these tests included fish from control groups C-I, C-II, and C-III, and the results were carefully analyzed for statistical differences between the fish that had been in zero-g and the fish that had remained on the ground at one-g. No differences were found; the fish that had been in zero-g behaved the same as the fish that stayed in one-g.

The flight fish seemed to dive frequently and to prefer the deep water in their tanks shortly after they arrived back on Earth. This observation was

checked 6 months later by timing each minnow for 2 minutes in a 500-milliliter glass cylinder filled with seawater. At that time, the Apollo hatchlings were about 35 millimeters long, the same size as the fish in the control groups. The percentage of time spent in the deeper half of the marked cylinder was 80 percent for the flight fish and 89 percent for the control group; that is, the flight hatchlings spent somewhat more time in *shallow* water than the ground-control fish did. Hatchlings from the eggs in Compartments 1 to 4 matched the control groups more closely; they spent about 90 percent of their time in the deeper water.

Several tests were made during short (25-second) periods of zero-g in airplanes to verify the looping observed on Apollo-Soyuz. Both the flight fish and the control fish looped in about the same way, which shows that the fish and embryos which had been on Apollo-Soyuz for 9 days had not retained any adaptation to zero-g. Instead of looping, some minnows "twisted" during these zero-g tests; that is, they would roll as they swam, searching for the proper belly-down position.

The average time from fertilization to hatching is about 21 days for *Fundulus heteroclitus* eggs. (There is a spread of hatching times in any batch of eggs: 21 days is the hatching time for 75 percent of the eggs.) Table 2.2

Time for 75 Percent of Eggs to Hatch **Table 2.2**

Compartment	Age at launch, hr	Hatch time, days			
		Flight	Control I[a]	Control II[b]	Control III[c]
1	32	15	23	26	21
2	66	15	23	26	21
3	128	15	25	27	24
4	216	18	19	19	22
5	336	23	23	24	25

[a]Control I: eggs flown to launch base and kept under simulated Apollo-Soyuz cabin conditions at one-g.

[b]Control II: eggs remaining at JSC in Houston in one-g.

[c]Control III: eggs 48 hours behind in development that were vibrated to simulate launch vibrations.

shows that the three youngest batches of eggs carried on Apollo-Soyuz hatched at 15 days, much sooner than the oldest batch in Compartment 5 and earlier than the control batches in one-g. Apparently, the development of young eggs was faster in zero-g.

The scientists dissected and examined eggs (embryos), hatchlings, and juvenile fish from both the flight and the control groups. The small otolith earstones were examined with an electron microscope, and no differences were found between the flight and control-group samples. Other parts of the developing embryos were also examined. The eyes, heart, nerves, and bones and the vestibula in the ear were found to be the same in the flight group as in the control group. There was no evidence of a calcium deficiency. Except for the shorter hatching time, the 9 days of zero-g seems to have had no effect on *Fundulus heteroclitus* minnows or their eggs.

D Questions for Discussion

(Fish in Zero-g)

1. There was no air in contact with the seawater where the MA-161 minnows lived for 9 days aboard Apollo-Soyuz, and no food was added. How would these factors affect their growth? In analyzing the experiment results, how was this effect avoided?

2. If it had been possible to put one of the flight minnows from Compartment 2 into a rotating striped drum on Apollo-Soyuz, how do you expect the minnow would have behaved?

3. How could zero-g accelerate the development of an embryo?

4. If the vestibular organs in a minnow were destroyed, what behavior would you expect of that minnow swimming in one-g? In zero-g?

3 Microbial Growth in Zero-g; Change in Biorhythm

Living organisms have many built-in periods or rhythms. The most obvious is the day-night circadian period of 24 hours. The purpose of the joint Experiment MA-147 was to discover whether spaceflight conditions, primarily weightlessness, would change a biorhythm that is not circadian—in this case, the rings produced by the microorganism *Streptomyces levoris*.

A Zone-Forming Fungi[2] Cultures

When *Streptomyces levoris* is "planted" in a uniform culture medium of agar, salts, glucose (sugar), and yeast, it grows outward and forms regular rings or zones of whitish spores, as shown in Figures 3.1 and 3.2. About 3 days after being planted at the center of a petri dish kept at 300 K (27° C, 81° F), the colony's rings are produced very regularly at a rate that depends on the culture medium. The medium used by the biologists at JSC gave a period close to 24 hours. (This is not the day-night circadian period; if a different culture medium were used, the rings would form at a different rate.) If the culture medium is uniform and the temperature is kept near 300 K, this formation of zones will continue for 2 or 3 weeks, although the period (time from one ring to the next) lengthens somewhat toward the end.

Between 30 and 40 cultures of *Streptomyces levoris* were prepared in this way both in Houston and in Baykonur (the Soviet launch base for Soyuz) 6 days before the Apollo-Soyuz mission. The U.S. and U.S.S.R. scientists tried to make these cultures exactly the same—to start them on a 24-hour period using the same culture medium and the same temperature. The starting time ("planting") was intentionally begun 12 hours earlier in the U.S.S.R. The idea was to exchange the two cultures in orbit to determine whether the U.S.S.R. spore rings would get "in step" with the U.S. spore rings in Houston after the flight and also whether the U.S. spore rings would later get in phase with the U.S.S.R. spore rings in Moscow. Unfortunately, the culture-medium materials used were not exactly the same in the two countries (the chemicals were obtained from different sources), and so the U.S.S.R. spore rings never matched the 24-hour period of the U.S. rings.

The scientists selected the best eight cultures in each country—four for the Apollo-Soyuz flight and four for the ground controls. All cultures were handled in the same way. The 60-millimeter petri dishes were packed in pairs in sealed boxes with glass covers, kept at 300 K in portable incubators before and after flight, and photographed every 12 hours. Figures 3.1 and 3.2 show that the zones formed regularly. As shown in Figure 3.3, the temperature did not remain constant during flight.

[2]BSCS-Y, Ch. 21.

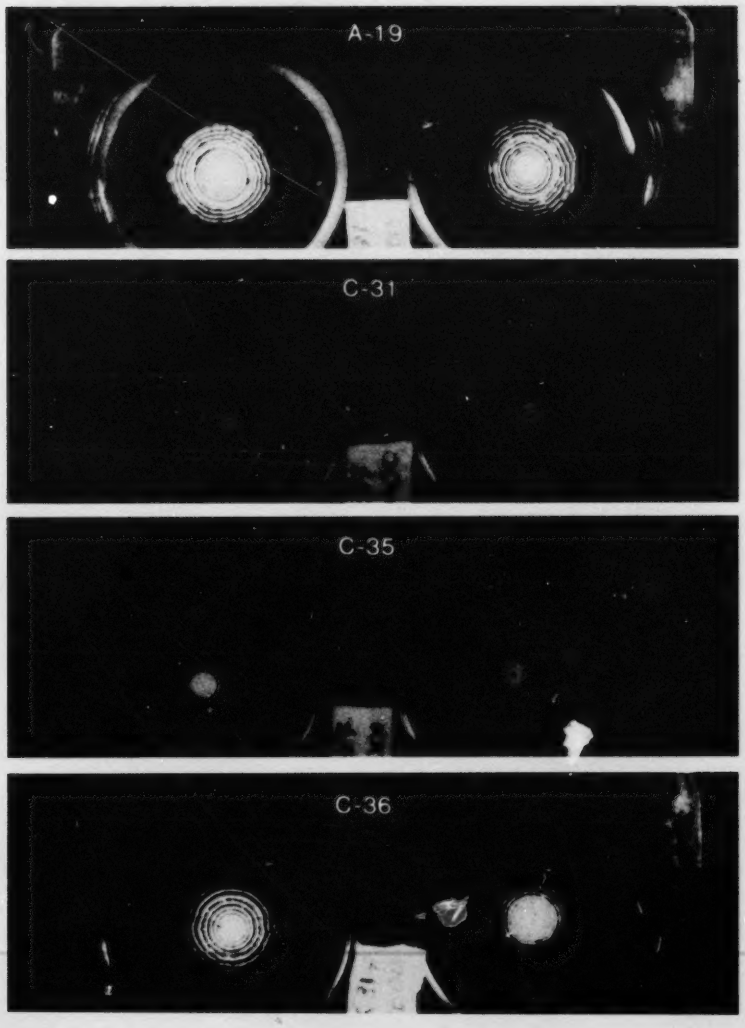
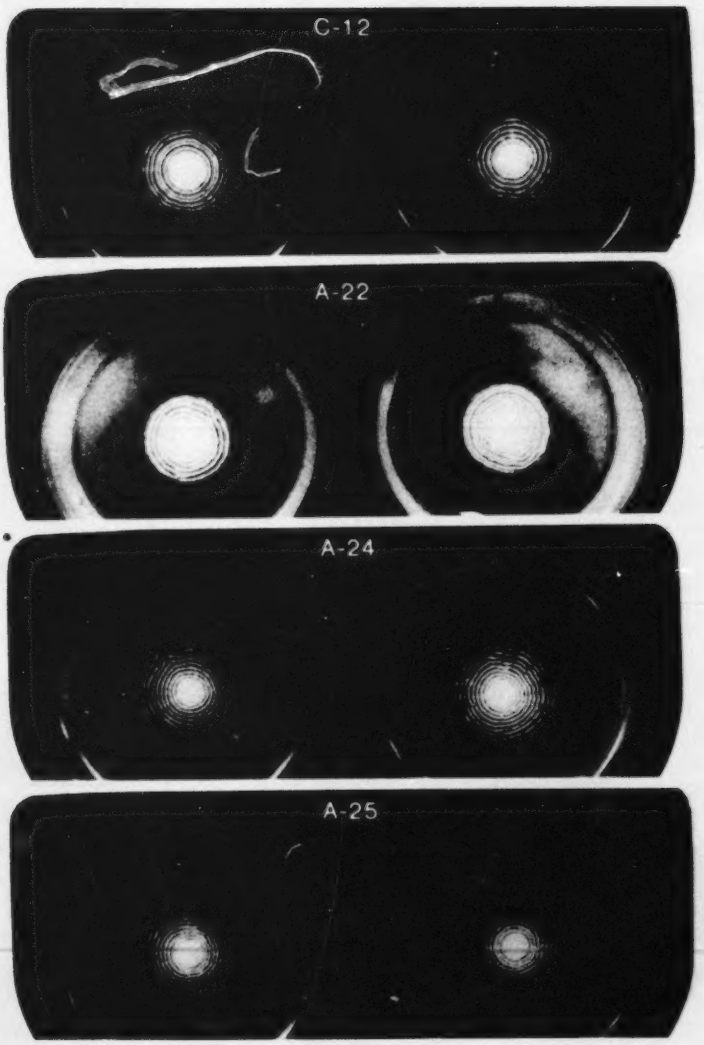


Figure 3.1 **Postflight photographs of *Streptomyces levoris* cultures in the U.S.S.R. The two petri dishes in Culture A-19 were launched in Apollo and recovered from Soyuz. Culture C-31 was both launched and recovered in Soyuz. Cultures C-35 and C-36 were Soviet ground controls.**



Postflight photographs of *Streptomyces levoris* cultures in the United States. The two petri dishes in Culture C-12 were launched in Soyuz and recovered from Apollo. Culture A-22 was both launched and recovered in Apollo. Cultures A-24 and A-25 were U.S. ground controls.

Figure 3.2

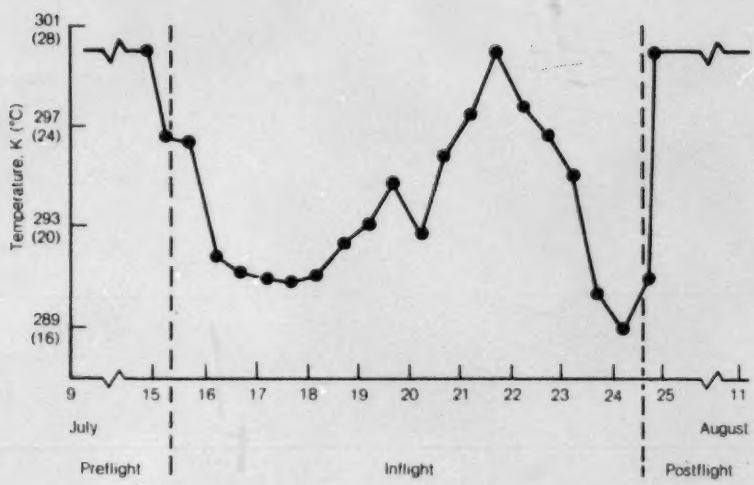


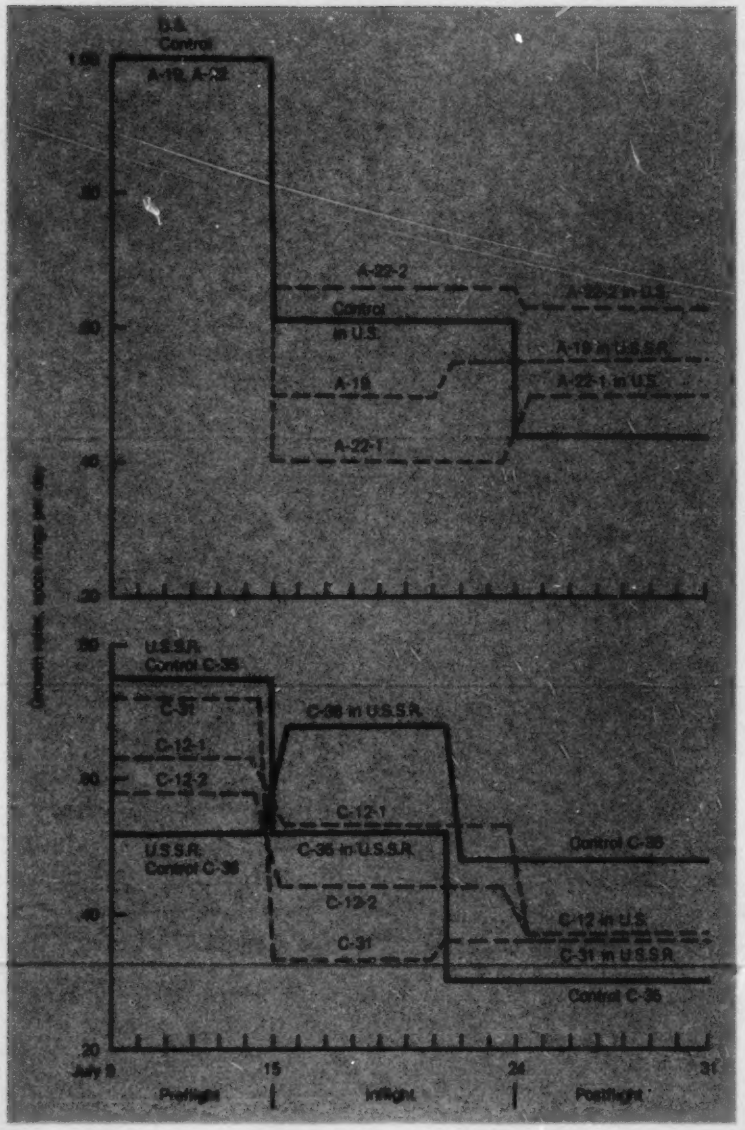
Figure 3.3 **Experiment MA-147 temperature changes during the flight. It had been intended to maintain incubation at 300 K (27° C, 81° F) throughout the flight.**

B Experiment MA-147 Results: Microbial Growth Rates

The scientists thought that cosmic rays passing through Apollo-Soyuz (see Pamphlet VI) might cause mutations or otherwise affect the microbial growth. Cosmic-ray detectors (special plastic films) were placed under the petri dishes and showed that many cosmic rays passed through them, but no evidence of mutagenic alterations of the *S. levoris* fungus was noticed.

From photographs like Figures 3.1 and 3.2, the number of rings could be counted and plotted against time. The *growth rate* is the number of rings (or fractions of a ring) added per day. These rates were averaged for three time intervals: the 6 days before launch, the 9 days in flight, and the 8 days following the Soyuz landing or Apollo splashdown. Figure 3.4 is a plot of these growth rates for 16 different cultures, the American (A numbers) at the top and the Soviet (C numbers) at the bottom. The control cultures are plotted by solid lines, the flight cultures by dashed lines.

The American cultures started nicely together at a 1.00-ring/day rate. The growth rates all decreased during the 9-day flight, although Culture A-22-2 dropped less than the others. In the postflight period, Cultures A-19-1, A-19-2, and A-22-1 increased their growth rates, whereas the U.S. control



Changes in *Streptomyces levoris* growth rates. Solid lines represent the ground controls; dashed lines represent the flight cultures.

Figure 3.4

cultures continued their decrease. The U.S.S.R. cultures had more erratic growth rates but showed an overall decrease.

The general conclusion from Figure 3.4 is that growth rates decreased aboard Apollo-Soyuz relative to the controls, both American and Soviet. This decrease could have been due to cosmic rays or weightlessness in the spacecraft, although the mechanism (how cosmic rays or zero-g could change the biorhythm) is not clear. Cultures A-19 and C-31-2 seem to have been affected by g-forces during splashdown, when Apollo decelerated at about 3 g's (see Pamphlet I). Figure 3.5 shows that double rings were formed that day in three of the four cultures on Apollo.

Although the investigators could not determine whether the spore rings in the cultures from the two countries would "get in step" (because the U.S. and U.S.S.R. growth rates were so different), Experiment MA-147 did show that the growth rate of *Streptomyces levoris* rings was reduced in zero-g. This result may give some clue to the effects of zero-g on living organisms.

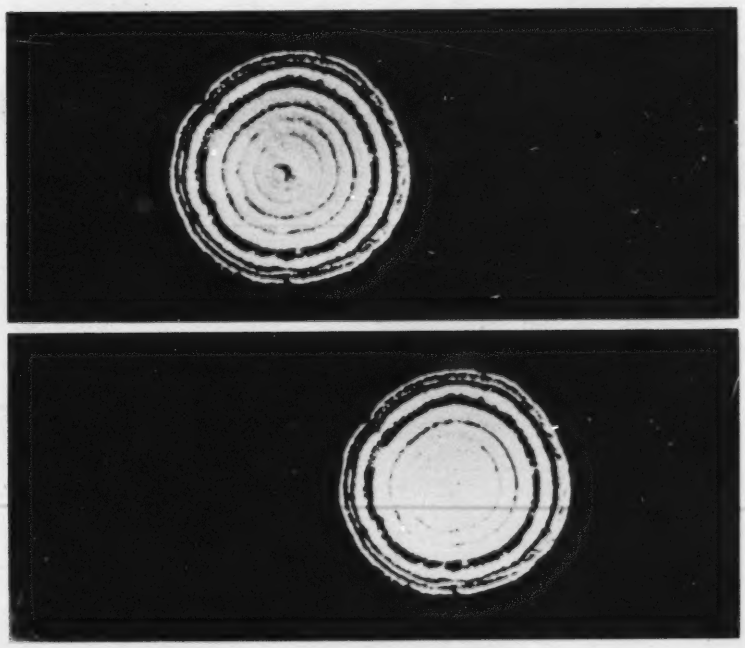


Figure 3.5 **Double rings of *Streptomyces levoris* spores. These rings were produced on the day of splashdown when Apollo decelerated at 3 g's.**

C Questions for Discussion

(Fungi, Spores)

5. As a colony of *Streptomyces levoris* grows across the culture medium, why should it produce spores periodically?

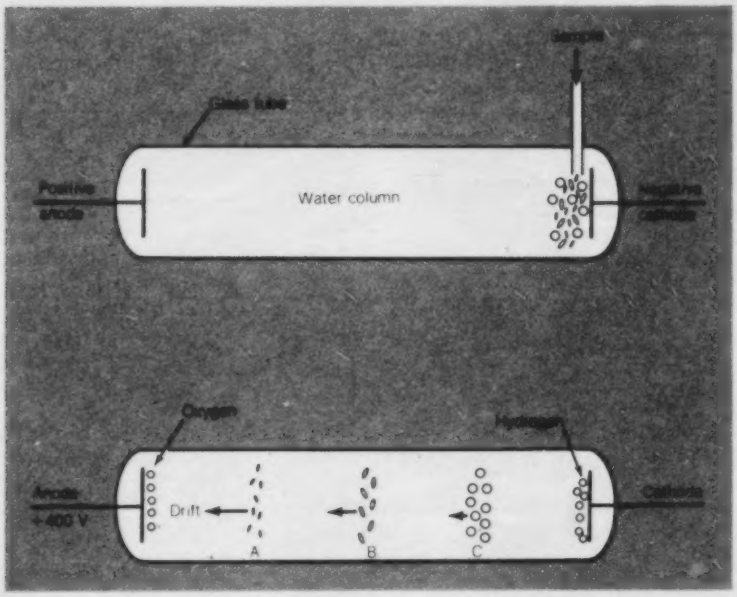
6. Why did the MA-147 investigators send so many cultures into orbit and maintain an equal number on the ground?

7. How might the effect of 3-g deceleration on spore rings of *Streptomyces levoris* be confirmed?

4 Separation of Cells by Electrophoresis

Living cells[3] in water are found to have a small negative electric charge on their surfaces. Different kinds of cells differ in three ways: (1) amount of electric charge, (2) size, and (3) shape. These differences have long been used by biologists to separate various kinds of cells from a mix by a process called electrophoresis—"electrical separation." The "electrophoresis column" is a water-filled glass tube with an electrode at each end (Fig. 4.1). The sample of mixed cells is inserted near the cathode (negative electrode), and a voltage is applied. Because of their negative charge, the cells are pushed away from the cathode toward the positive anode at the other end of the column.

For a given voltage, the speed at which the cells drift through the water (the "electrophoresis mobility") depends on each cell's electric charge, size, and



Static-column electrophoresis and separation bands. At the start (top sketch), all cells in the sample are near the cathode. After a time (bottom), three different types of cells are separated because type A drifts more than B, and type C drifts less than either A or B.

Figure 4.1

[3]BSCS-Y, Ch. 6; BSCS-B, Ch. 13.

shape. It is very nearly the same for cells of the same type. Therefore, as shown in Figure 4.1, cells of type A eventually drift farther down the column than do cells of type B. Cells of type C (those with lowest electrophoretic mobility) drift least. The sample mix of cells is thus separated in the electrophoresis column, and any one type of cell (a pure component of the original mix) can be drawn from the band in which it is concentrated. If opaque cells or colored cells (like red blood cells) are present, a photograph of the column will show the width of each band and the approximate fraction of cells in each band.

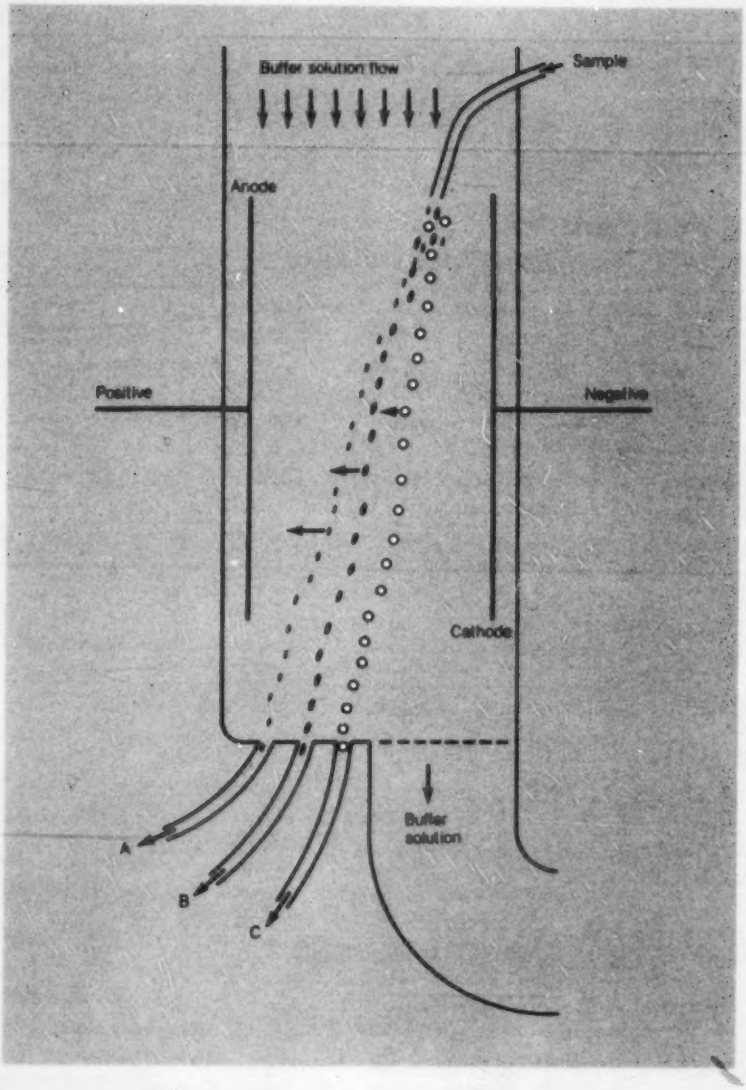
A "static column" is shown in Figure 4.1. The buffer solution (water and various salts) is at rest and the cells drift to form slowly moving bands. If the purpose is to separate large numbers of cells for medical experiments or for the manufacture of medicines, it would be more effective to separate different cell types across a *flowing* buffer solution, as shown in Figure 4.2. In this "free-flow electrophoresis," each cell type would be collected continuously from a certain place in the flowing buffer solution after drifting across the stream to that location. This technique requires a very smooth flow with no bubbles, eddies, or crosscurrents in the buffer solution.

A Difficulties Caused by Gravity

There are practical difficulties with both free-flow and static-column electrophoresis. One difficulty is that the buffer solution is separated by electrolysis and produces oxygen and hydrogen at the electrodes. These gases must be withdrawn from the column so that the bubbles will not disturb the drift of the cells or the electric field along the column. In the weightless condition aboard a spacecraft, there is no force of gravity (zero-g), and the bubbles don't rise as they do in one-g on the ground—they stay at the electrodes.

Two other difficulties become important when biologists in one-g try to separate cells of almost the same electrophoretic mobility. For instance, two kinds of kidney cells important in modern medicine drift at so nearly the same rate that their bands (Fig. 4.1) overlap, even when the column is very long. The bands are not sharp (narrow) because of unwanted disturbance (currents) in the water. Even if a static column is kept perfectly still, when the voltage is turned on, an electric current flows, heats the water, and causes convection currents in the buffer solution. In zero-g, there is no convection, so the heat does not start water currents and the electrophoresis bands are sharper than in one-g on the ground.

The second difficulty in one-g is sedimentation. All cells have a higher density than water and tend to sink or "settle out" under the force of gravity.



Free-flow electrophoresis. The electric field, from right to left, is across the (downward) flow of the buffer solution. This causes type A cells in the sample to move farthest left; type C cells move least toward the left. The three types can be collected by the three tubes (A, B, and C) at the bottom.

Figure 4.2

CS 30

In zero-g, there is no such tendency, and the only force on a cell is the electrical one, which produces the separation.

For these reasons, two electrophoresis experiments were performed on Apollo-Soyuz. Static electrophoresis columns had been tried on two previous NASA missions to the Moon, Apollo 14 in 1971 and Apollo 16 in 1972, but neither one worked well.

B Experiment MA-011, Electrophoresis Technology

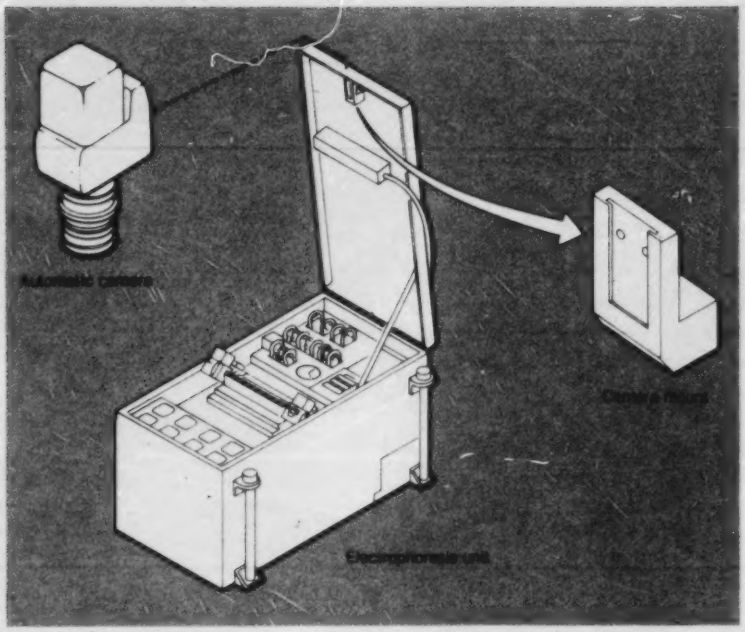
The experiences on the Apollo 14 and 16 missions showed the scientists at MSFC that the static electrophoresis column must be designed very carefully so that samples could be inserted accurately and all gases could be removed. To achieve the best electrophoresis separation that can be accomplished in zero-g, eight different samples were to be separated and photographed and the columns frozen so that the bands could be examined later on Earth. Four of the samples were artificial mixes of known proportions of red blood cells from a rabbit, a human, and a horse; two were lymphocytes from a human; and two were the two important types of human kidney cells mentioned in Section 4A.

The static electrophoresis unit shown in Figure 4.3 was designed so that each 15-centimeter (6-inch) long column of water could be plugged into it. The astronaut took a sample from a freezer and pushed it into a slot on the side of a tube made of Lexan plastic so that it was centered in the 6-millimeter (0.25-inch) wide column of buffer solution. He set a switch for the desired separation time (45, 60, or 75 minutes). The electrophoresis unit then automatically turned on 400 volts, controlled the static-column temperature at 278 K (5° C, 41° F), photographed the column every 3 minutes, disposed of hydrogen and oxygen gases safely, and finally froze the column (to 233 K, −40° C, −40° F). Then the astronaut put the frozen column in the freezer for return to Earth and plugged in the next column. One of the plug-in static columns is shown in Figure 4.4.

C Experiment MA-011 Results

Columns 1, 3, 4, and 7 worked well, Column 7 (the kidney cells) best of all. However, the electric power in Column 2 failed after 3 minutes, and some thin tubes used to remove gas from the buffer solution became clogged on Columns 5 and 6. Failures like these are to be expected in any scientific experiment.

When the film and frozen columns were returned to the scientists at MSFC, the film was developed and examined. Figure 4.5 shows the separation of red



MA-011 electrophoresis unit with camera. An electrophoresis column is plugged in between electrodes in the center of the box. The camera is attached to the open box top for automatic photographs of the column.

Figure 4.3

blood cells (rabbit, human, and horse, from left to right). The frozen columns were then sliced and melted, and the cells were recovered from each slice. About half the red blood cells were still alive. Because the tubes got clogged in Column 6, the buffer solution became too basic (pH factor less than 2.5) and only 1 percent of the lymphocytes were still alive.

Column 3 contained a mix of human kidney cells, which cannot be separated in one-g on Earth. All the kidney cells in this column were alive, and the separation was checked by growing cultures of the cells from slices of the frozen column near the separation bands. The type of kidney cell needed produces urokinase, an enzyme used to treat people suffering from blood-clot conditions. The cells from one slice produced more than four times as much urokinase as did the original kidney-cell mix. This demonstration showed that electrophoresis in zero-g can separate kidney cells of very nearly the same type. The separation of urokinase-producing kidney cells in zero-g may become one of the important medical manufacturing technologies of the future.

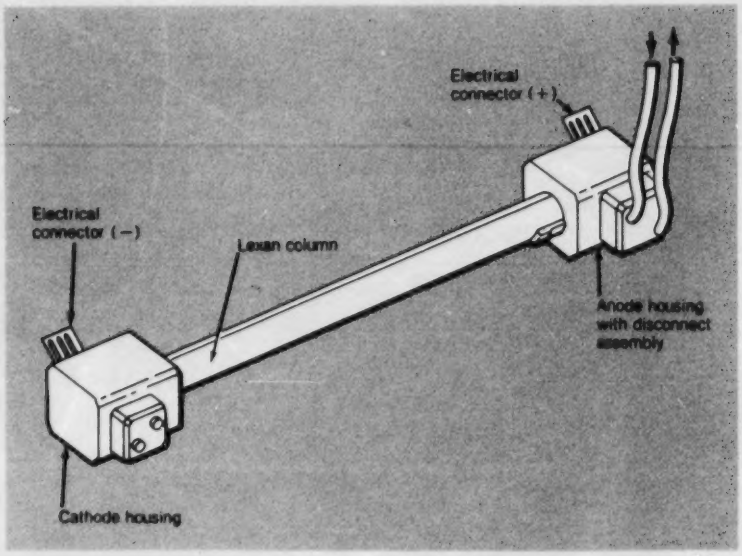


Figure 4.4 **Electrophoresis plug-in static column for the MA-011 Experiment. The transparent Lexan column allows the separation bands to be photographed.**

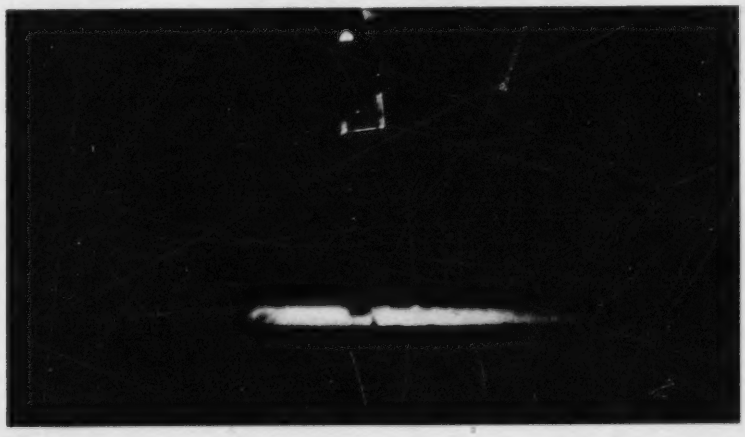


Figure 4.5 **Flight photograph of MA-011 Column 5. The mix of red blood cells has separated into three well-defined bands: rabbit (left), human (center), and horse (right).**

D Experiment MA-014, Free-Flow Electrophoresis

A schematic diagram showing how free-flow electrophoresis should work is given in Figure 4.2. The concept was developed by Kurt Hannig, the German Principal Investigator for Experiment MA-014. Free-flow electrophoresis should lead to rapid separation of large amounts of biological material in a continuous process. The outlets at the bottom of Figure 4.2 should drain off separately most of the cells of types A, B, and C, and the rest of the buffer solution is pumped around to the top to be recycled through the tube.

The actual MA-014 equipment on Apollo-Soyuz did not have these outlets for cell types A, B, and C. Instead, a beam of light was directed through a slit at the bottom of the flow tube to 128 photometers along a line at the top. These photometers scanned the strip of light from the anode side to the cathode side and showed how the amount of light was reduced by cells moving downward near the bottom of Figure 4.2.

Four different samples were prepared just before the Apollo launch and kept refrigerated at 277 K (4° C, 40° F) until the astronauts operated the MA-014 experiment on July 16, 1975, for almost 85 minutes. Everything seemed to work properly, and the photometer scans were recorded on magnetic tape that was returned to Earth. When the MA-014 scientists in Munich, Germany, played back these tapes, they found that the light had been too bright, which reduced the accuracy of the measurements.

The measurements of free-flow electrophoresis separation of three of the samples are shown in Figure 4.6. (The fourth scan of a mix of human and rabbit erythrocyte cells was ruined by the bright light.) The separations of the bone-marrow cells, spleen cells, and lymph-node cells mixed with human erythrocytes are considerably better than attempted separations in one-g on the ground. These results show that free-flow electrophoresis in spacecraft in zero-g may become as valuable a technology as static electrophoresis.

E Questions for Discussion

(Electrophoresis)

8. The water (buffer solution) in a static electrophoresis column (Fig. 4.1) should be perfectly still. What about thermal motion of the water molecules?

9. When the voltage is turned off in a static electrophoresis column, as in Figure 4.1 (bottom), what happens?

10. The two closely similar types of kidney cells were not perfectly separated by the MA-011 electrophoresis column. Explain how the separation could have been improved by a second run. Could multiple runs be used in free-flow electrophoresis?

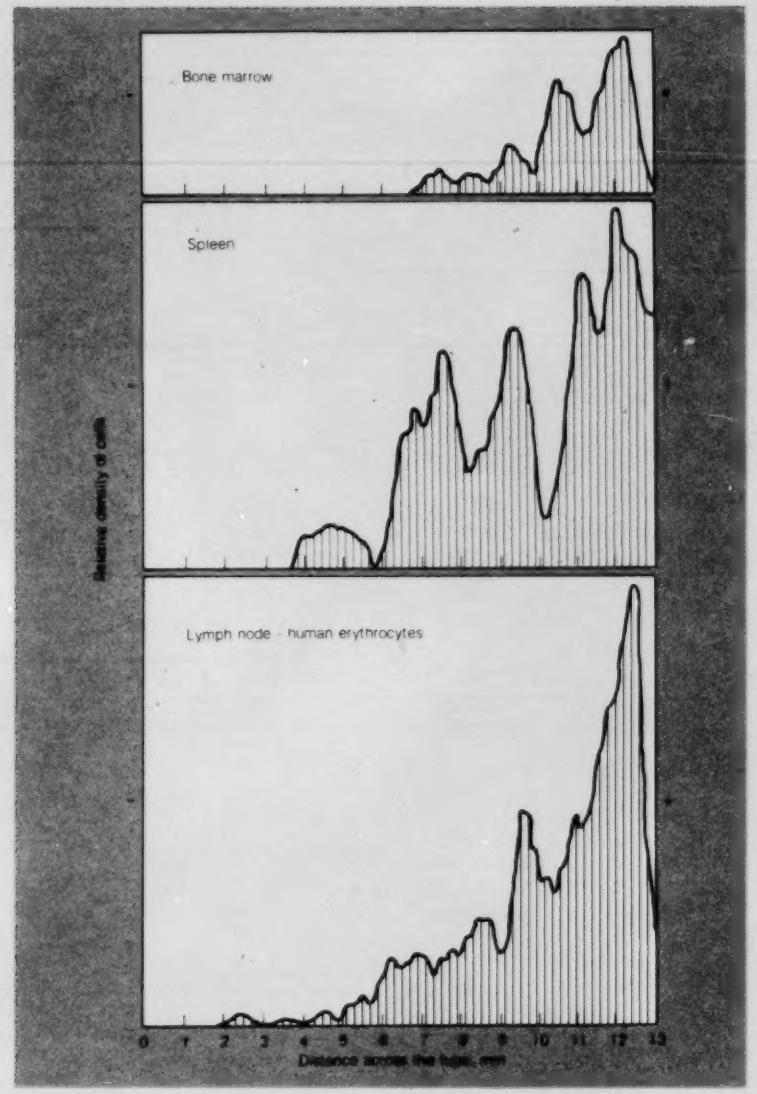

Bone marrow
Spleen
Lymph node - human erythrocytes
Relative density of cells
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Distance across the tube, mm

Figure 4.6 **MA-014 free-flow separation in zero-g. The darkness of cells at different places in the free-flowing buffer solution is plotted against the distance between the electrodes.**

5 Microbes at Large in the Spacecraft

Several studies of microbes[4] in spacecraft and on crewmembers had been made in the United States and the U.S.S.R. before the Apollo-Soyuz mission. As a result of the American studies, astronauts were examined by physicians before their missions and were isolated for the last 3 weeks before launch so that they would not be exposed to unfamiliar disease germs.

However, the human body carries many microbes with it—in the intestinal tract, nose, and ears and on the hair and skin. It would be impossible, and unwise, to eliminate all these microbes before flight. The Apollo and Soyuz spacecraft were not sterilized, and various kinds of microbes were carried aboard by the crews. The question was whether the *balance* between microbes and man would be changed within the confines of the spacecraft during flight. There might be a change in the *numbers of microbes*, or in the *crewmen's resistance* (immunity) to disease, or in the microbes' *power to infect*. Also, microbes brought up from Russia by a cosmonaut might be *transferred* to an astronaut in Apollo-Soyuz, or vice versa.

The earlier studies indicated that microbes were transferred between crewmembers during flight. These studies also resulted in two broad and different ideas: (1) that only a few types of aerobic microbes (floating in the cabin "air") would survive in flight and they would produce large populations, and (2) that crewmembers might suffer "microbic shock" on return to Earth after adjusting to fewer microbes in the spacecraft cabin; that is, the crewmembers might get sick from the microbes normally all around us on Earth.

A Joint Experiment, AR-002, Microbial Exchange

A large number of biologists in the United States and the U.S.S.R. prepared a detailed plan to measure precisely which microbes were launched in Apollo-Soyuz in and on each astronaut and cosmonaut. The scientists wanted to determine whether microbes were transferred between crewmembers during flight or increased in any crewmember. They also wanted to test for possible changes in crewmembers' resistance to disease during flight. Medical tests extended from 45 days before the 9-day flight to 30 days afterward. The tests included taking samples of blood and saliva and "swab samples" from the hair, ears, nose, mouth, and throat and from five areas on the skin of each astronaut and cosmonaut. Similar samples were taken from the five backup crewmembers before the flight.

The type of swab used in the AR-002 Experiment is shown in Figure 5.1. It was sealed before and after use and was soaked in a fluid that keeps microbes

[4]BSCS-Y, Chs. 22, 23.

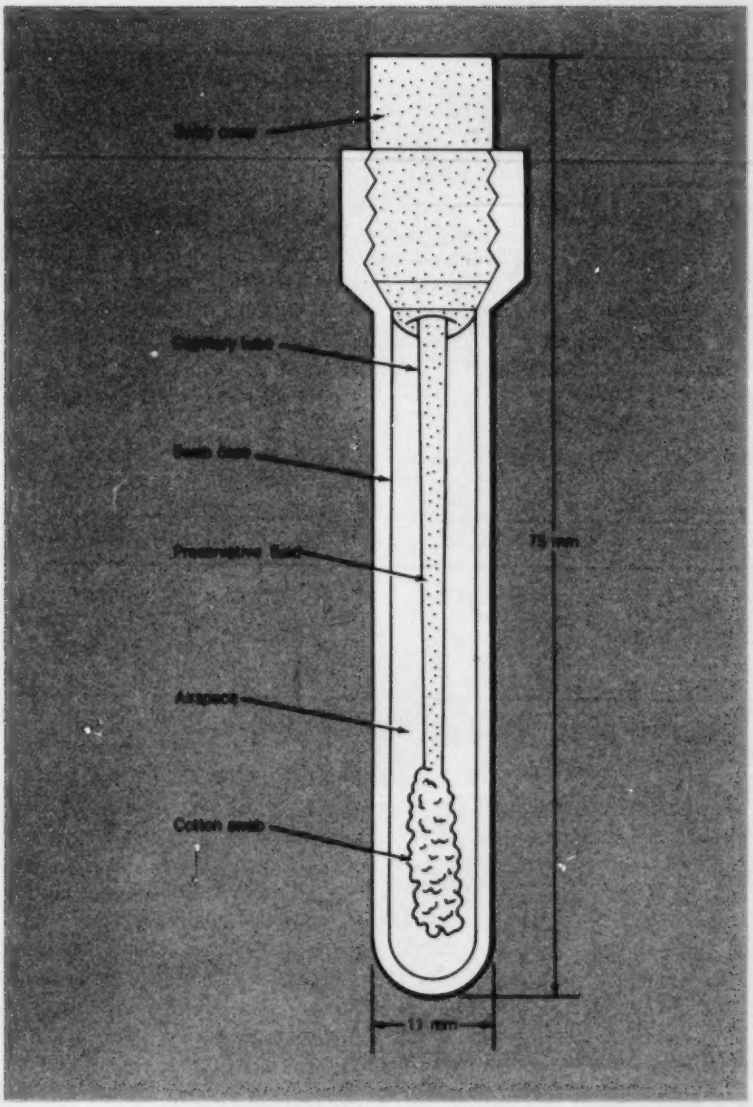


Figure 5.1 **The AR-002 swab. Eighty of these swabs were carried on Apollo-Soyuz. Each swab was sealed in a plastic tube before and after use. Preservative fluid in the stem of the swab kept the microbes on the swab alive.**

alive. During flight, each crewmember used his own set of 10 swabs to rub the specified places on his body. (This had been done before the flight under the supervision of a scientist, who made sure that the astronaut understood how to take the samples by himself.) Two other sets of 15 swabs each were used to "mop up" microbes from 15 specific surface areas in the Apollo spacecraft and 15 in the Soyuz spacecraft, each marked to be exactly 100 square centimeters. All these swabbings were taken during the same hour on July 18, 1975, and the 80 swabs were returned to Earth by Soyuz and carried to Moscow for immediate analysis.

The tests were numerous and complex. They were as nearly as possible exactly the same as the tests performed on the ground before and after flight. In this way, the biologists obtained fairly accurate measurements of the numbers and kinds of microbes in various places at several different times. For example, Figure 5.2 shows the numbers of aerobic bacteria on the skin-swab samples from the five crewmembers at nine different times. (The "quantitation" plotted in Figures 5.2 and 5.3 is the logarithm of the number of bacteria from 1 square centimeter of the surface swabbed. "Quantitation 5," for example, means that about 100 000 bacteria were swabbed from each square centimeter of Astronaut Stafford's skin—the average on his five skin swabs. There is a fair amount of scatter in these bacterial counts. However,

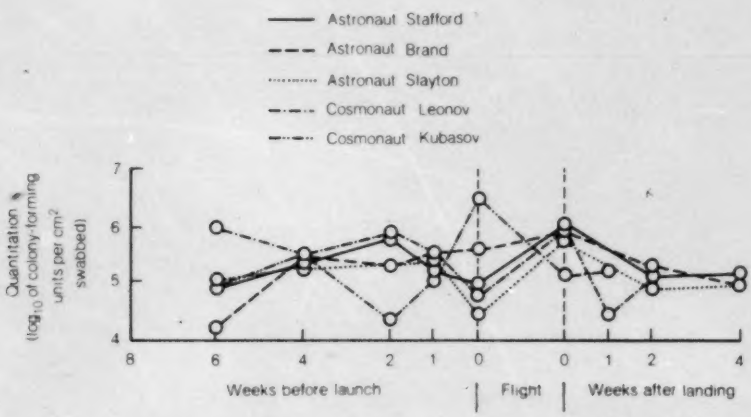


Figure 5.2 **Number of bacteria in skin swabs from the five Apollo-Soyuz crewmen. Quantitation is the logarithm of the number of bacteria per square centimeter of skin swabbed. The number varied from about 10^4 to more than 10^6. (Log 10^4 = 4; log 10^6 = 6.)**

because all the swabs were used and analyzed in the same way, the average quantitation shows *changes* in the number of bacteria as accurately as possible.)

B Experiment AR-002 Results

Counts of several different kinds of microbes were measured in the swabs. Gram-negative rods of the genus *Haemophilus* are generally found in the human mouth and were detected in the astronauts' throat swabs and gargle water. The yeast *Candida albicans,* which may cause "thrush" (boils and sores in the mouth), was found in two crewmembers' mouths. *Staphylococcus aureus,* which can cause boils, is sometimes found in the nose and occasionally on the skin; it was found on all crewmembers.

Before and after the flight, measurements were also made on saliva and blood samples in order to detect specific immunity of the crewmembers to disease. Two factors measured in the saliva samples were Immunoglobulin A (IgA) and lysozyme. The IgA is a collection of different kinds of proteins, some of which are antibodies and each of which attacks one specific kind of bacteria. (Antibodies give a person immunity[5] to a disease after he has had the disease and recovered from it.) Lysozyme is a chemical (enzyme) that kills or weakens all bacteria. Astronaut blood samples were tested for immunity to six kinds of bacteria and to the yeast *Candida albicans.* The results are expressed in terms of the dilution of blood serum that barely allows the microbes to grow in a culture (stronger serum would kill them). "Dilution 8" means only half the immunity of "dilution 16."

The results of Experiment AR-002 were based on these measurements: (1) bacteria counts from swab samples before, during, and after flight; (2) IgA and lysozyme measurements in saliva samples before and after flight; and (3) immunity to seven types of microbes measured in blood samples before and after flight.

Plots like the one in Figure 5.2 show that the number of bacteria on the skin of any one crewmember changed considerably with time. This finding is typical and was expected. Measurements of bacteria in the mouth, nose, and throat varied even more. However, the average count of all five crewmembers was fairly constant during the 84 days covered by the measurements. There was clearly *no change* during or immediately after the Apollo-Soyuz flight.

Surfaces inside the spacecraft were swabbed immediately before and after as well as during the flight. The three sets of measurements of aerobic bacteria

[5]BSCS-Y, Ch. 23

are plotted for each spacecraft in Figure 5.3. As expected, some parts of the spacecraft were cleaner than others, but the averages (open circles) show significant changes. The bacteria brought in with the astronauts increased the spacecraft bacteria count. The postflight drop in the bacteria count was caused by an accidental intake of jet fuel when a valve was opened to let in outside air during splashdown (see Pamphlet I). This gas, a mixture of dinitrogen tetroxide and nitrous oxide, burned the eyes, lungs, and bare skin of the astronauts and partly sterilized the Apollo cabin. In the Soyuz spacecraft, the general trend of cabin bacteria was upward by about a factor of 10, as in previous flights.

Measurements of specific microbes in the crewmembers also revealed little change. Figure 5.4 shows the results on gram-negative *Haemophilus* cells in the astronauts' mouths and throats. The average dropped slightly during the isolation period before launch but was not greatly affected by the flight. The same can be said for *Candida albicans* in the mouth and *Staphylococcus*

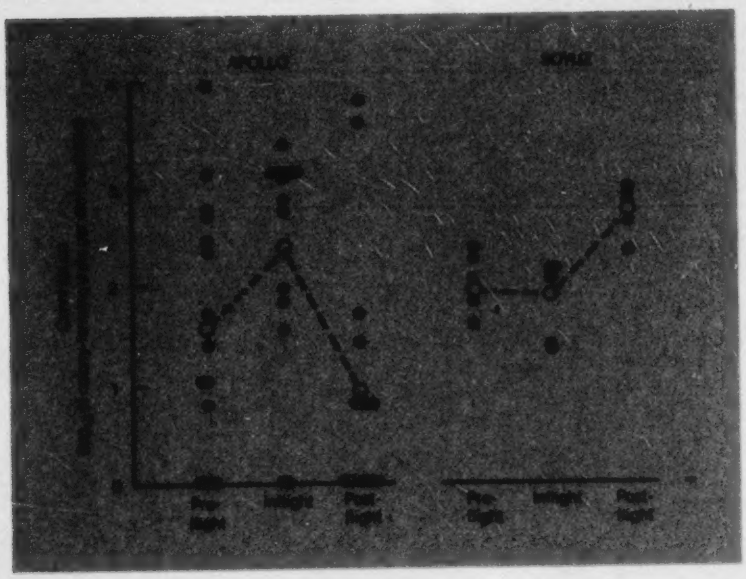


Figure 5.3

Number of bacteria in swabs from exposed surfaces on the spacecraft. There were large differences between different surfaces in Apollo—from less than 10 to about 10^4 bacteria per square centimeter. The open circles show the average of all surfaces on each of three dates.

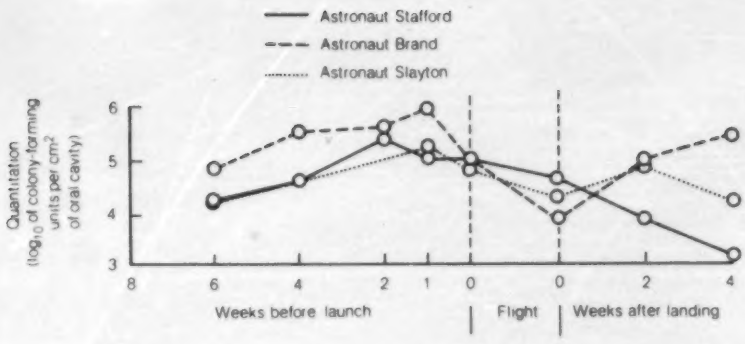


Figure 5.4 **Number of *Haemophilus* cells in astronauts' oral cavities. The numbers varied from a high of 10^6 down to less than 10^4 bacteria per square centimeter. These variations are normal in the mouths of Americans.**

aureus in the nose and on the skin. There were 13 slightly different strains of *S. aureus* among the five crewmembers. Bacteria of one of these strains, together with *C. albicans,* were transferred from one astronaut to another during the flight. There was no transfer of microbes between astronauts and cosmonauts during the 2 days they were together. Moreover, all these microbial measurements do *not* support the idea that just a few types of microbes would multiply rapidly during spaceflight and the other types would die off.

The idea that astronauts' immunity to disease would change during spaceflight was not confirmed for the 6- to 9-day Apollo-Soyuz flight. The measured amounts of IgA and lysozyme in saliva showed no general changes, although there was a normal amount of variation. The most accurate measurements of immunity were made on the blood samples of all five crewmembers. The dilution of blood serum, which is a measurement of immunity, was very different for each crewmember for the various bacteria tested. For instance, Astronauts Stafford and Slayton had dilutions of 1024 against *Haemophilus influenzae* and Astronaut Brand and Cosmonaut Leonov had dilutions of 8192 against *Bacillus abortus*. All crewmembers had dilutions of about 8 for *S. aureus* and *C. albicans*. Two large changes were observed; however, in the other 38 cases of crewmembers' immunity versus bacterial counts, there seemed to be no change in immunity during or after the Apollo-Soyuz flight.

C Questions for Discussion

(Microbes, Immunity)

11. What would the astronauts notice if there were a large increase in the number of aerobic microbes inside their spacecraft?

12. When explorers first visited isolated Eskimo tribes in the Far North, the Eskimos caught colds and many died. How do you explain this?

13. Why would it be unwise to sterilize a spacecraft thoroughly and eliminate all microbes on the hair and skin and in the ears, nose, and mouth of each astronaut before launch?

14. If there were a tendency for some types of microbes to multiply in a spacecraft and for other types to die off, how could a stable population be artificially maintained?

15. If the balance of microbes is not maintained during a long spaceflight, what problem could later arise?

6 Changes in Astronaut Immunity During Spaceflight

Human blood carries several types of cells[6] and chemicals that fight disease. The simplest of these is the white blood cell or *leukocyte* that is formed in bone marrow and then carried around the body in the blood stream. During its short life of only a few hours, the leukocyte can "eat up" (ingest) bacteria. It can move through small blood vessels, cling to the wall near an infection, and squeeze between tissue cells to reach a cut or wherever the enemy bacteria are. Biologists can watch this process through a microscope. They can count the leukocytes and estimate the percentage that are able to ingest bacteria.

Lymphocytes are cells that are formed in the lymph glands as well as in bone marrow. Several types exist, including T-cells, which adhere to "foreign" cells in the blood, and B-lymphocytes, which manufacture immunoglobins (IgG, IgM, IgA, IgD, and IgE), the proteins that make up antibodies.

A Experiment MA-032, Polymorphonuclear Leukocytes

White blood cells were studied in seven blood samples taken from each of the three astronauts from 30 days before to 30 days after the Apollo-Soyuz mission. (None was taken during the flight.) Because leukocytes are replaced in the bloodstream every few hours, the samples taken immediately after splashdown were the most important. These samples, analyzed on the recovery ship U.S.S. *New Orleans*, contained leukocytes formed during flight in zero-g.

Counts showed between 4000 and 14 000 leukocytes/mm^3 of blood. Differences as high as 6000 were found between the counts of the three astronauts at any one time, and a change of 400 leukocytes/mm^3 in one astronaut's blood was noted during the 69-day period. Similar variations are found in all humans, so the MA-032 scientists concluded that the Apollo-Soyuz flight caused no marked changes in leukocyte counts.

More detailed tests were made on leukocytes that had been separated from the blood samples in a test tube. The cells from the splashdown sample were found to have about the normal ability to cling to blood-vessel walls and the normal migration (moving ability). (These qualities were measured in a new type of test that shows the clinging and mobility in glass tubes rather than in actual blood vessels.) Some of the leukocytes are polymorphonuclear (PMN) cells, known to be the most active in attacking bacteria. They can be recognized in a microscope by a large, irregular nucleus which can be seen after

[6]BSCS-Y, Ch. 11; BSCS-B, Ch. 13.

they are stained. (See cover photograph.) The fraction of PMN leukocytes was higher than normal in the splashdown blood samples from the astronauts. The leukocytes were tested for ability to ingest bacteria *(Staphylococci)* and found to be normal.

The Principal Investigator concluded that the experiment showed no change in leukocyte function (abilities) immediately after splashdown, although the leukocyte abilities might have been different 4 or 5 hours earlier, during flight.

B Experiment MA-031, Cellular Immune Response

Experiment MA-031 was based on astronaut blood samples similar to those used in Experiment MA-032, and the white-blood-cell counts agreed fairly well. Experiment MA-031 studied the lymphocytes separated from the blood in a centrifuge using a special liquid that provides a density gradient. This liquid is put in a test tube and the blood sample added on top. Then the test tube is placed in the centrifuge and spun around. The higher density blood cells are pulled far down into the liquid by centrifugal action, while the more buoyant lymphocytes remain near the center, all at one level. When the test tube is removed from the centrifuge, the liquid and lymphocyte cells are sucked out by pipette. Counts showed an average of 2700 lymphocytes/mm^3 before flight, 2000/mm^3 just after splashdown, and 2900/mm^3 later. More than half of these were T-lymphocytes, a quarter were B-lymphocytes, and the rest were "nonreactive." In the splashdown blood samples, the nonreactive lymphocytes were far fewer; otherwise, the counts showed no significant change. These measurements were different from those taken after the 84-day Skylab 3 mission, when B- and T-lymphocytes were lower in number following splashdown.

The Principal Investigator tested the abilities of the lymphocytes to function by mixing them with four different antigens.[7] About 10^5 lymphocytes were mixed with each antigen in a liquid medium. The antigens were phytohemagglutinin, pokeweed mitogen, Concanavalin A, and influenza virus. They were incubated for 3 or 5 days at 310 K (37° C, 100° F) and then "fed" radioactive thymidine. Thymidine is one of four "code" substances in deoxyribonucleic acid[8] (DNA). It was made radioactive by substituting tritium (^3H) for ordinary hydrogen (^1H) in the thymidine molecule. This

[7] BSCS-Y, Ch. 23.
[8] BSCS-Y, Ch. 28; BSCS-B, Ch. 12.

^{3}H-thymidine then served as a radioactive tracer in the DNA made by the lymphocytes. After 2 hours in the incubator at 310 K, the lymphocytes were washed onto filters and their radioactivity was measured. The more reactive lymphocytes had used more ^{3}H-thymidine to make DNA, and this gave a higher radioactive count rate.

The results show a definite decrease in lymphocyte ability to react with phytohemagglutinin just after splashdown and a day later. The Concanavalin A ability remained the same on all except Astronaut Stafford's lymphocytes, where it was *strengthened* at splashdown. The pokeweed mitogen and influenza virus results fluctuated and showed no consistent pattern.

C Questions for Discussion

(Leukocytes and Lymphocytes)

16. If you are suddenly scared, your glands release the hormones adrenalin and cortisone in your blood. What then happens to the white-blood-cell count (number of leukocytes per cubic millimeter) in your blood?

17. How are PMN leukocytes distinguished from other white blood cells?

18. Just after splashdown, the astronauts' blood samples showed high white-blood-cell counts and low lymphocyte counts. What might account for this?

Appendix A

Discussion Topics (Answers to Questions)

1. (Sec. 2D) If the minnows had been completely sealed in a liter of water, they would have died because of lack of oxygen. However, the polyvinyl plastic "breathes"; that is, oxygen, carbon dioxide, and water vapor pass through the thin plastic bags. Incoming oxygen from the Apollo cabin atmosphere refreshed the water, and exhaled carbon dioxide was released to the cabin, together with a small amount of water vapor. The lack of food would slow the minnows' growth, but the control groups were not fed either. Hence, the effects of not being fed were the same on the flight fish and the control groups of fish.

2. (Sec. 2D) The minnows in Compartment 2 had been preconditioned with vertical stripes in their tanks. If the lighting were uniform, one of these minnows would probably swim around next to one of the stripes as if the drum were vertical (Fig. 2.1).

3. (Sec. 2D) The lack of gravitational force might allow more rapid blood flow and more rapid chemical reactions, both of which would accelerate embryo development.

4. (Sec. 2D) A minnow without vestibular organs would probably depend on its eyes and swim with its back toward the light, both in one-g and in zero-g. It would not loop in zero-g if there were a steady light to provide orientation.

5. (Sec. 3C) The *Streptomyces levoris* colony "expects" its food to run out after 12 to 15 hours' growth along the surface. Therefore, it produces spores that can start a new colony after being blown by the wind to some other place with more food.

6. (Sec. 3C) Not all the microbes of one species like *Streptomyces levoris* behave exactly the same. The combined results from four to eight cultures would be more reliable than the results from any one culture. The ground controls were necessary to show the *difference* in average behavior between zero-g and one-g.

7. (Sec. 3C) Placing several cultures of *Streptomyces levoris* in a low-speed centrifuge for 1 to 5 minutes while a spore ring is in the process of formation might show the effect of Apollo's 3-g deceleration before splashdown.

8. (Sec. 4E) Thermal motions in the buffer solution of a static electrophoresis column smear the separation bands slightly. This smearing is small compared to the effect of convection currents.

9. (Sec. 4E) When the voltage is turned off on a static electrophoresis column, the separation bands stop moving. They can be observed for several minutes but slowly dissipate because of convection currents and thermal motion (diffusion).

10. (Sec. 4E) If a frozen slice of the cells in one separation band (Fig. 4.1) of kidney cells (together with the buffer solution) is melted, there is an admixture of some unwanted cells from the nearby separation band. However, the proportion of the wanted cells has been greatly increased over the original sample. Using this melted slice as the sample in a second run, the frozen slice of the next separation band will contain a higher proportion of the wanted cells. Similar improvement can be achieved in free-flow electrophoresis by using the outflow at "A" in Figure 4.2 for the sample in a second run.

11. (Sec. 5C) A large increase in aerobic microbes in the spacecraft could result in an unpleasant odor in the cabin atmosphere and in molds growing on exposed surfaces.

12. (Sec. 5C) The Eskimos' immunity to certain bacteria and viruses carried by the explorers was low because they had not yet been exposed to those particular microorganisms. The explorers and trappers were usually resistant (immune) to the microbes they carried.

13. (Sec. 5C) Sterilizing an astronaut (or any animal) is virtually impossible. Even partial sterilization of astronauts and spacecraft would be unwise because it would destroy the balance among microbes, man, and man's immunities. After such an attempt, the astronauts' immunities could slowly wear off, and they could catch many diseases on returning to Earth.

14. (Sec. 5C) If one type of microbe tends to die off, cultures of that microbe could be carried in incubators on the spacecraft and released from time to time. Just before such releases, the overpopulated species could be reduced by cleaning exposed surfaces.

15. (Sec. 5C) If the balance of microbes were not maintained on a long spaceflight, the astronauts could lose their immunity to the microbes that died out and become more vulnerable to those microbes on returning to Earth.

16. (Sec. 6C) The white-blood-cell count increases because the hormones cause changes in the blood vessels. Leukocytes from the small blood vessels enter the main bloodstream.

17. (Sec. 6C) The PMN leukocytes form one of five classes of white blood cells. They are recognized by their complex nuclei when viewed in a microscope after they have been stained with dyes.

18. (Sec. 6C) Blood tests at the conclusion of previous space missions all showed the same effect. Biologists think that spaceflight somehow causes an increase in white-blood-cell count and a decrease in the lymphocyte count. The excitement of returning to Earth may account for the increase in white blood cells. The corticosteroid treatment that was administered to the Apollo-Soyuz astronauts just after splashdown because of their painful exposure to jet fuel in the Command Module during reentry probaby accounts for the prolonged reduction in lymphocytes.

Appendix B

SI Units
Powers of 10

International System (SI) Units

Names, symbols, and conversion factors of SI units used in these pamphlets:

Quantity	Name of unit	Symbol	Conversion factor
Distance	meter	m	1 km = 0.621 mile 1 m = 3.28 ft 1 cm = 0.394 in. 1 mm = 0.039 in. 1 μm = 3.9×10^{-5} in. = 10^4 Å 1 nm = 10 Å
Mass	kilogram	kg	1 tonne = 1.102 tons 1 kg = 2.20 lb 1 gm = 0.0022 lb = 0.035 oz 1 mg = 2.20×10^{-6} lb = 3.5×10^{-5} oz
Time	second	sec	1 yr = 3.156×10^7 sec 1 day = 8.64×10^4 sec 1 hr = 3600 sec
Temperature	kelvin	K	273 K = 0° C = 32° F 373 K = 100° C = 212° F
Area	square meter	m^2	1 m^2 = 10^4 cm^2 = 10.8 ft^2
Volume	cubic meter	m^3	1 m^3 = 10^6 cm^3 = 35 ft^3
Frequency	hertz	Hz	1 Hz = 1 cycle/sec 1 kHz = 1000 cycles/sec 1 MHz = 10^6 cycles/sec
Density	kilogram per cubic meter	kg/m^3	1 kg/m^3 = 0.001 gm/cm^3 1 gm/cm^3 = density of water
Speed, velocity	meter per second	m/sec	1 m/sec = 3.28 ft/sec 1 km/sec = 2240 mi/hr
Force	newton	N	1 N = 10^5 dynes = 0.224 lbf

Quantity	Name of unit	Symbol	Conversion factor
Pressure	newton per square meter	N/m^2	$1\ N/m^2 = 1.45 \times 10^{-4}$ lb/in^2
Energy	joule	J	1 J = 0.239 calorie
Photon energy	electronvolt	eV	$1\ eV = 1.60 \times 10^{-19}$ J; $1\ J = 10^7$ erg
Power	watt	W	1 W = 1 J/sec
Atomic mass	atomic mass unit	amu	$1\ amu = 1.66 \times 10^{-27}$ kg

Customary Units Used With the SI Units

Quantity	Name of unit	Symbol	Conversion factor
Wavelength of light	angstrom	Å	$1\ Å = 0.1\ nm = 10^{-10}$ m
Acceleration of gravity	g	g	1 g = 9.8 m/sec^2

Unit Prefixes

Prefix	Abbreviation	Factor by which unit is multiplied
tera	T	10^{12}
giga	G	10^{9}
mega	M	10^{6}
kilo	k	10^{3}
hecto	h	10^{2}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}

Powers of 10

Increasing	Decreasing
$10^{2} = 100$	$10^{-2} = 1/100 = 0.01$
$10^{3} = 1\,000$	$10^{-3} = 1/1000 = 0.001$
$10^{4} = 10\,000$, etc.	$10^{-4} = 1/10\,000 = 0.000\,1$, etc.
Examples:	Example:
$2 \times 10^{6} = 2\,000\,000$	$5.67 \times 10^{-5} = 0.000\,056\,7$
$2 \times 10^{30} = 2$ followed by 30 zeros	

Appendix C

Glossary

References to sections, Appendix A (answers to questions), figures, and tables are included in the entries. Those in *italic* type are the most helpful.

aerobic microbes those microbes capable of living in the presence of oxygen; anaerobic microbes are killed by oxygen. (Secs. 5 to 5B; App. A, no. 11; Figs. 5.2, 5.3)

anode a positively charged plate or wire in a vacuum tube or electrophoresis column. (Secs. 4, 4D; Figs. 4.1, 4.2, 4.4)

antigen a substance that causes the production of antibodies by lymphocytes in the human body. (Sec. 6B)

Apollo-Soyuz a joint U.S.-U.S.S.R. mission from July 15 to July 24, 1975. Apollo, the three-man U.S. spacecraft, consisted of the Command Module (CM) connected to the Service Module (SM) and the Docking Module (DM). For 2 days, the DM was attached to Soyuz, the two-man Soviet spacecraft. The two spacecraft were in a circular orbit inclined 51.8° to the Equator, with a 93-minute period, 222 kilometers above the Earth's surface. See Pamphlet I.

AR-002 the joint experiment, Microbial Exchange, on the Apollo-Soyuz mission. (Secs. 1, *5A, 5B;* Figs. 5.1 to 5.4)

backup crew a crew trained to replace the prime crew if necessary. There were three astronauts and two cosmonauts in the backup crews trained and ready to substitute for Apollo and Soyuz crewmembers in case any crewmember got sick or was injured before launch so that he could not make the flight. (Sec. 5A; Fig. 5.4)

band the "separation band" in an electrophoresis static column in which all the cells of one type move along the column. (Secs. 4 to 4B; App. A, nos. 8 to 10; Figs. *4.1*, 4.5)

buffer solution the liquid (water with several grams of various salts per liter) through which cells are moved by electrical forces in electrophoresis. (Secs. *4*, 4A to 4E; App. A, nos. 8, 10; Figs. 4.2, 4.6)

Candida albicans a yeast-type cell sometimes found in the human mouth. (Sec. 5B)

cathode a negatively charged plate or wire in a vacuum tube or electrophoresis column. (Secs. 4, 4D; Figs. 4.1, 4.2, 4.4)

centrifuge a device that swings material around in a circle. The centrifugal force on this material can be made to equal many g's. A high-speed centrifuge can separate cells in a liquid according to the density of each kind of cell. (Sec. 6B; App. A, no. 7)

control group a group of individuals (spores, eggs, microbes, fish) with the same characteristics as the experimental group and handled in the same way except for the one factor (such as time in zero-g) that is being tested by the experiment. (Secs. 2A, 2C, 3A, 3B; App. A, nos. 1, 5; Figs. 3.1, 3.2, 3.4; Table 2.2)

convection the up-and-down drafts in a fluid or gas heated from below in one-g. Because the density of the heated fluid is lowered, the fluid rises. After cooling at the top, its density increases, and it sinks. (Sec. 4A; App. A, nos. 8, 9)

cosmic ray an extremely high speed ion coming from outer space. See Pamphlet VI. (Secs. 1, 3B)

culture a colony of cells grown on a nutrient medium under controlled conditions in an incubator. The *culture medium* provides food for the cells; it is usually a thin layer of jelly made from agar, sugar, water, and other materials. (Secs. 3A, 3B, 4C; App. A, nos. 6, 14; Figs. 3.1, 3.2, 3.4)

DNA (*deoxyribonucleic acid*) the chemical compound in the nuclei of living cells that carries genetic information used when the cell reproduces. (Sec. 6B)

Docking Module (DM) a special component added to the Apollo spacecraft so that it could be joined with Soyuz. (Sec. 2B) See *Apollo-Soyuz* and Pamphlet I.

electrophoresis the separation of cells in a liquid by an electric field or a voltage. (Secs. 1, *4*, 4A to 4E; App. A, nos. 8 to 10; Figs. *4.1*, *4.2*, 4.3 to 4.6)

electrode an electrically charged plate or wire in a vacuum tube or electrophoresis column. (Secs. 4, 4A; Fig. 4.6)

embryo a living organism before birth or hatching. An embryo slowly develops all the structures of the full-grown organism. (Secs. 2, 2A, 2C; App. A, no. 3; Table 2.1)

free flow a form of electrophoresis in which the buffer solution and the sample flow uniformly between the electrodes. The sample, a mixture of cells inserted near the cathode, drifts sideways across the buffer solution and is collected at several places downstream. (Secs. 1, *4*, 4A, 4D; App. A, no. 10; Figs. *4.2*, 4.6)

Fundulus heteroclitus the species of minnow (commonly called killifish) used in the MA-161 Experiment. See *killifish*.

g-force the force of gravity; on the Earth's surface, it is one-g. In orbit, there is no force (zero-g). When a spacecraft is accelerated during launch or decelerated during reentry, everything inside it experiences a force that may be as high as 3 g's. (Sec. 3B; App. A, nos. 3, 7)

Haemophilus a genus of microbes. Several species are found in and on humans. (Sec. 5B; Fig. 5.4)

hatchling a newborn minnow, just after breaking out of the egg casing. (Secs. *2*, 2A to 2C) See *killifish*.

IgA (Immunoglobulin A) a protein which contains antibodies that attack specific species of microbes in human blood. (Secs. *5B*, 6)

immunity the ability of the human body to kill specific types of invading microbes. (Secs. 1, 5, *5B*, 6, 6B; App. A, nos. 12, 13, 15) See *lymphocytes*.

JSC the NASA Lyndon B. Johnson Space Center in Houston, Texas.

juvenile a minnow 2 to 4 weeks old, about one-third as large as a full-grown fish; used in the MA-161 Experiment. (Secs. *2*, 2A to 2C) See *killifish*.

killifish the small minnows *(Fundulus heteroclitus)* used in the MA-161 Experiment. (Secs. 1, *2*, *2A*, 2B, *2C;* App. A, nos. 1 to 4; Figs. 2.1, 2.2; Tables 2.1, 2.2) See *embryo, hatchling, juvenile*.

KSC the NASA John F. Kennedy Space Center at Cape Canaveral, Florida, where the Apollo spacecraft was launched on July 15, 1975.

leukocytes white blood cells in human blood. The PMN leukocytes are particularly effective in fighting microbes in the body. Leukocytes are manufactured in bone marrow. (Secs. 1, *6*, 6A; App. A, nos. 16, 17)

Lexan a polycarbonate plastic from which the static electrophoresis columns used in the MA-011 Experiment were made. (Sec. 4B; Fig. 4.4)

$\log_{10}$ logarithm to base 10. $\text{Log}_{10}\ 10 = 1$; $\log_{10}\ 100 = 2$, $\log_{10}\ 100\ 000 = 5$, and so on. (Sec. *5A;* Figs. 5.2 to 5.4)

lymphocytes cells in human blood and saliva that provide specific immunity to different types of diseases (bacterial and viral). They are manufactured in the lymph nodes and bone marrow. (Secs. 4B, 4C, 6, *6B*, 6C; App. A, no. 18)

lysozyme an enzyme in the blood and other body fluids that kills or weakens bacteria. (Sec. *5B*)

MA-011 the Electrophoresis Technology Experiment on the Apollo-Soyuz mission. (Secs. 1, *4B*, *4C*, 4E; App. A, no. 10; Figs. 4.3 to 4.5)

MA-014 the Electrophoresis Experiment. (Secs. 1, *4D;* Fig. 4.6)

MA-031 the Cellular Immune Response Experiment. (Secs. 1, *6B*)

MA-032 the experiment concerning the Effects of Spaceflight on Polymorphonuclear Leukocyte Response. (Secs. 1, *6A*, 6B)

MA-147 The Zone-Forming Fungi Experiment. (Secs. 1, *3*, 3A, 3B; Figs. 3.1 to 3.5)

MA-161 the Killifish Hatching and Orientation Experiment. (Secs. 1, 2, *2A*, *2B*, 2C, 2D; App. A, nos. 1, 2; Figs. 2.1, 2.2; Tables *2.1*, 2.2)

microbes microscopic organisms, some of which cause disease. Many microbes are found in and on the human body. (Secs. 1, *5*, 5A, 5B; App. A, nos. 6, 11 to 15; Fig. 5.1)

mitogen a nonspecific substance (like a plant seed) that causes lymphocytes to produce antibodies. (Sec. 6B)

mutation a change in the DNA "code," usually caused by a cosmic ray passing through the nucleus of a cell that controls development. The developed organism, a mutant, differs from others in its species. (Sec. 3B)

otoliths the three small calcium "earstones" in the vestibular organ of the ear. (Secs. 2, 2A, 2C) See *vestibula*.

petri dish a shallow dish of glass or plastic used to hold a culture medium. (Secs. 3A, 3B; Figs. 3.1 to 3.3)

photometer an instrument that uses electrical voltage to measure the intensity (brightness) of light. (Sec. 4D)

PMN leukocytes *poly*morpho*nuclear* cells, the most active type of white blood cells. (Secs. 1, *6A;* App. A, no. 17)

Principal Investigator the individual responsible for a space experiment and for reporting the results.

quantitation a number that best represents the statistical results of biological measurements involving many individuals. (Sec. *5A;* Figs. 5.2 to 5.4)

radioactive tracer a chemical compound containing radioactive isotopes of an element which can be followed through complex biological processes by counting the radioactive disintegrations of the isotope. (Sec. 6B)

saliva the liquid in the human mouth secreted by glands back of the lower molars. It contains water, digestive chemicals, and lymphocytes. (Secs. 5A, 5B)

Skylab a very large space workshop that NASA put into orbit on May 14, 1973. It was visited by three astronaut crews who worked on scientific experiments in space for a total of 172 days. (Secs. 2, 2A, 6B)

spore a small seed-germ that can grow into a microbe or a plant such as a fern. Spores of *Streptomyces levoris* were observed in Experiment MA-147. (Secs. 3A, 3B; App. A, nos. 5, 7; Figs. 3.4, 3.5)

Staphylococcus aureus a type of bacteria generally found in the nose and on the skin of humans. (Sec. *5B*)

static column the motionless liquid containing a sample mix of cells to be separated by electrophoresis. (Secs. 1, *4*, 4A to 4C, 4E; App. A, nos. 8 to 10; Figs. *4.1*, 4.3 to 4.5)

sterilize to kill all (or almost all) microbes in and on an object, usually by high temperature or fumigation with poisonous chemicals. (Secs. 5, 5B; App. A, no. 13)

Streptomyces levoris a funguslike microorganism used in the MA-147 Experiment. (Secs. 3, *3A*, 3B; App. A, nos. 5 to 7; Figs. 3.1, 3.2, 3.4, 3.5)

swab a wad of cotton used in the AR-002 Experiment to collect microbes from crewmembers' hair, skin, ears, nose, and throats and from surfaces in the spacecraft. (Secs. *5A*, 5B; Figs. 5.1 to 5.3)

vestibula the organ in the ear that provides a sense of "which way is down" (along the force of gravity). It is thus responsible for the sense of balance—the "vestibular sense." (Secs. 2, 2A to 2C; App. A, no. 4; Table 2.1)

weightlessness the condition of free fall or zero-g, in which objects in a spacecraft are weightless. (Secs. 1, 2, 3, 3B, 4A) See *zero-g*.

zero-g the condition of free fall and weightlessness. When there are no forces on objects in a spacecraft, they are "in zero-g." (Secs. 1, 2, 2A to 3, 3B to 4B, 4D; App. A, nos. 4, 6; Fig. 4.6)

Appendix D

Further Reading

Is This Your Day?–The Biorhythm Bible by George Thommen, Avon Press (New York), 1976—how mathematical applications are used to describe the biological and sociological scheme of things; biorhythm and how it works.

Living in Space by M. R. Sharp, Doubleday & Co., Inc. (New York), 1969—a well-illustrated survey of space biology that describes weightlessness.

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